Application No.: 09/258600 Docket No.: CPI-012CP4DV

## **AMENDMENTS TO THE SPECIFICATION**

Please replace the last paragraph on page 54 as follows:

GPA<sub>ID</sub> and GPA<sub>LW</sub> hybrids. The regions of high homology among Ga subunits that have been identified by sequence alignment are interspersed throughout the molecule. The G1 region containing the highly conserved -GSGESGDST- (SEQ ID NO:127) motif is followed immediately by a region of very low sequence conservation conservation, the "i1" or insert 1 region. Both sequence and length vary considerably among the i1 regions of the  $G\alpha$  subunits. By aligning the sequences of Ga subunits, the conserved regions bounding the i1 region were identified and two additional classes of GPA1-Ga hybrids were constructed. The GPA<sub>ID</sub> hybrids encode the amino terminal 102 residues of GPA1 (up to the sequence -QARKLGIQ-(SEQ ID NO:122)) fused in frame to mammalian Gα subunits, while the GPA LW hybrids encode the amino terminal 244 residues of GPA1 (up to the sequence -LIHEDIAKA- (SEQ ID NO:123) in GPA1). The reason for constructing the GPA<sub>ID</sub> and GPA<sub>LW</sub> hybrids was to test the hypothesis that the i1 region of GPA1is required for mediating the interaction of GPA1 with yeast GBy subunits, for the stable expression of the hybrid molecules, or for function of the hybrid molecules. The GPA<sub>ID</sub> hybrids contain the amino terminal domain of GPA1 fused to the i1 domain of mammalian subunits, and therefore do not contain the GPA1 i1 region, while the GPA LW hybrids contain the amino terminal 244 residues of GPA1 including the entire i1 region (as defined by sequence alignments). Hybrids of both GPA ID and GPALW classes were constructed for GaS, Gai2, Gai3, Gao, and Ga16; none of these hybrids complemented the *gpa1* growth arrest phenotype.

Please replace the last paragraph on page 55 and the first paragraph on page 56 as follows:

Gas Hybrids. There is evidence that the "switch region" encoded by residues 171-237 of  $G\alpha$  transducin (using the numbering of Noel et al (1993)) also plays a role in  $G\beta\gamma$  coupling. First, the G226A mutation in G $\alpha$ S (Miller et al. 1988) prevents the GTP-induced conformational change that occurs with exchange of GDP for GTP upon receptor activation by ligand. This residue maps to the highly conserved sequence -DVGGQ- (SEQ ID NO:128), present in all Ga subunits and is involved in GTP hydrolysis. In both the Gat and Gai1 crystal structures, this sequence motif resides in the loop that connects the  $\beta$ 3 sheet and the  $\alpha$ 2 helix in the guanine nucleotide binding core. In addition to blocking the conformational change that occurs upon GTP binding, this mutation also prevents dissociation of GTPliganded Gas from Gby. Second, crosslinking data reveals that a highly conserved cysteine residue in the  $\alpha 2$  helix (C215 in G $\alpha$ 0, C210 in G $\alpha$ t) can be crosslinked to the carboxy terminal region of Gβ\_subunits. Finally, genetic evidence (Whiteway et al. 1993) identifies an important single residue in GPA1 (E307) in the  $\beta2$  sheet of the core structure that may be in direct contact with By. A mutation in the GPA1 protein at this position suppresses the constitutive signalling phenotype of a variety of STE4 (GB) dominant negative mutations that are also known to be defective in Gα-Gβγ association (as assessed in two-hybrid assay in yeast as well as by more conventional genetic tests).

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Please replace the paragraph in the middle of page 98 as follows:

Clearly, these sequences encode novel peptides, as the native a-factor sequence differs considerably:

Tyr lle lle Lys Gly Val Phe Trp Asp Pro Ala (SEO ID NO: 129).

Please replace the full paragraph on page 110 as follows:

A hybrid gene encoding the prepro-region of human POMC (accession # K02406; Takahashi, H., et al (1983) Nucleic Acids Research 11:6847-6858) and the coding region of a single repeat of mature α-factor will be constructed in the following fashion. The preproregion of human POMC will be amplified with an HindIII site at the 5' end and a BbsI site at the 3' end using VENT polymerase and the following primers: 5' GGGAAGCTT ATGCCGAGATCGTGCCAGCCGC 3' (SEQ ID NO:30) (HindIII site is underlined and initiation codon italic bold) antisense is and GGGGAAGACTTCTGCCCTGCGCCGCTGCTGCC 3' (SEQ ID NO:31) (BbsI recognition is underlined), leaving the amino acid sequence -SSGAGOKR- (SEQ ID NO:125) at the 3' end with a Bbs1 site leaving an overhang at the -KR- dibasic cleavage sequence. The coding region of  $\alpha$ -factor will be amplified from Cadus 1219 with a Bbs1 site at the 5' end and a BglII site at the 3' end using the primers 5' GGGGAAGACCCGCAGGAGGCAGAAGCTT GGTTGCAG (BbsI NO:32) site is underlined) GGGAGATCTTCAGTACATTGGTTGGCC 3' (SEQ ID NO:33) (BglII site is underlined, termination codon is bold). The PCR fragment encoding the pre-pro segment of POMC is restricted with HindIII and BbsI and gel purifed, the PCR fragment encoding α-factor is cut with BbsI and BglII and gel purified, and Cadus 1215 is cut with BglII and partially with HindIII and the HindIII-BglII restricted vector containing the pAlter polylinker sequences is gel purified. Three-part ligation of the two PCR products with HindIII and BglII digested Cadus 1215 will yield a hybrid POMC/α-factor gene in which the first 104 amino acids residues are from POMC and the remaining 17 are from α-factor. The structure of this hybrid gene around the PC1 cleavage site is: -RNSSSSGSSGAGOKREAEAWHWLQLKPGOPMY\* (SEQ ID NO:34) where residues donated by POMC are underlined, the dibasic cleavage site is underlined bold, and the sequence of mature α-factor is in italics. The tetrapeptide -EAEA-(SEO ID NO:130) juxtaposed between the dibasic cleavage site and the amino-terminal tryptophan of mature α-factor should be removed by the dipeptidyl aminopeptidase activity of ste13p.

Please replace the first line of Table 7 on page 153 as follows:

GPA1 RIDTTGITETEFNIGSSKFKVLDAGGQRSERKKWIHCFEGITAV LFVLAMSEYDQMLFEDER (SEQ ID NO:131)